DEPMEDS LABORATORY PROCEDURES DEPARTMENT OF CLINICAL SUPPORT SERVICES U.S. ARMY MEDICAL DEPARTMENT CENTER AND SCHOOL FORT SAM HOUSTON, TEXAS 78234-6137

MCCS-HCL STANDING OPERATING PROCEDURE 01 November 01

SICKLEDEX TEST

1. PRINCIPLE:

Sickledex tube test is a qualitative tube test for the determination of Hemoglobin S. The reaction is based on the relative insolubility of Hemoglobin S when combined with buffer and a reducing agent. When Sickledex reagent powder is mixed with Sickledex test solution and whole blood is added, blood containing Hemoglobin S will form a cloudy, turbid suspension. Other hemoglobins are more soluble and will form a transparent solution when tested.

2. SPECIMEN:

Fresh whole blood from a finger puncture or blood mixed with anticoagulants including EDTA, sodium or potassium oxalate, sodium citrate, ACD, CPD, CPDA-1 solutions, or heparin may be used.

3. REAGENTS AND EQUIPMENT:

- a. Add the entire contents of one vial of Sickledex reagent powder to one bottle of Sickledex test solution. *Open powder vials must be used in reconstitution immediately or discarded.*
- b. Shake vigorously for 10 seconds.
- c. Date the reconstituted Sickledex test solution on the bottle. The test solution may be used for 60 days when stored tightly capped and at 2 to 8°C. Dispenser caps must be closed during storage.

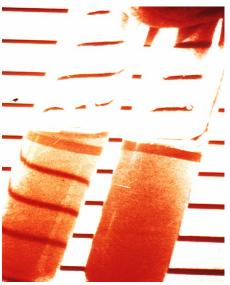
4. PROCEDURE:

a. Dispense 2 mL of reconstituted Sickledex test solution into one test tube. (12x75 clear glass) The test solution must be at room temperature (20-30°C) for testing purposes.

- b. Add 0.02mL of whole blood or control specimen to the tube. When specimens from severely anemic individuals are tested (7.0 grams hemoglobin or less), deliver 0.04 mL of blood to the test tube
- c. Mix the contents of the tube thoroughly by inverting several times. Allow the test tube to stand in the test tube holder for a minimum of 6 minutes at room temperature (20-30°C). The interpretation of results may be performed at any time between six and fifteen minutes after the addition of the blood sample.

4. RESULTS:

- a. The reaction is read macroscopically by looking through the tube at the ruled lines on the test tube holder.
- b. A positive test for Hb-S is indicated by a cloudy turbid suspension through which the ruled lines behind the test tube are not visible.
- c. A negative test is indicated by a transparent suspension. The ruled lines behind the tube are visible.



POSITIVE EXAMPLE SHOWN ON THE RIGHT

NEGATIVE EXAMPLE SHOWN ON THE LEFT

- 5. Limitations of the procedure
 - a. Coarse flocculation in the test tube may occur if the test specimen contains abnormal elevations of total serum protein, particularly the gamma globulins, and may be incorrectly interpreted as positive for Hb-S. When this occurs, the specimen should be washed once with normal saline and centrifuged. The supernate is then decanted and the red cells re-suspended to the original volume with normal group compatible serum and re-tested.
 - b. False negatives may occur if the concentration of Hb-S is below the level of sensitivity of Sickledex. Low levels of Hb-S are frequently observed in infants

younger than three months of age. It is recommended therefore, infants not be tested prior to six months of age.

6. INTERPRETATION:

- a Negative the person does not have Hgb S
- c. Positive perform hemoglobin electrophoresis to confirm the results